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FIRST APPLICATION OF THE TRANSPLACENTAL MICRONUCLEUS TEST IN WILD RODENTS (***)

(*Mammalia*)

Abstract. — In a research carried out to evaluate the effects of pesticides on wild murine populations, the embryos and fetuses of *Mus domesticus* and *Apodemus sylvaticus* from agricultural areas of the Rome neighbours (Maccarese and Tolfa) were examined. The analysis of the ossification centers in order to determine the fetal age and the transplacental micronucleus test to evaluate mutagenetic risk to which they are exposed, were carried out. The results show that prenatal damages can be induced; thus the importance to apply the micronucleus test to wild murine populations is confirmed.

Riassunto. — *Prima applicazione del test dei micronuclei transplacentare in Roditori selvatici.*

Nell'ambito di una ricerca condotta per valutare gli effetti di pesticidi su popolazioni naturali di murini sono stati presi in esame embrioni e feti di *Mus domesticus* e di *Apodemus sylvaticus* provenienti da aree agricole della provincia di Roma (Maccarese e Tolfa); sono stati eseguiti l'analisi dei centri di ossificazione per determinare l'età di feti e il test dei micronuclei per valutare il rischio mutagenetico al quale essi sono sottoposti. I risultati ottenuti indicano la possibilità di induzione di danni prenatali; pertanto è confermata l'importanza di estendere l'applicazione di test di mutagenesi a popolazioni naturali di murini.

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Introduction.

The present work is a part of a research carried out in agricultural, urban and nuclear power plant areas with the aim to evaluate some critical environment situations; the survey was carried out on wild murine populations utilized as biological indicators, applying different methods (mutagenetic, biometric and histopathological analyses and radiometric determinations) to detect the effects of environmental pollutants.

In this work the risk from the pesticide exposure in wild murine populations (*Mus domesticus, Apodemus sylvaticus*) was studied in two sites of the Rome neighbours: the reclaimed land of Maccarese with wheat, maize and vegetables cultivations regularly treated with pesticides (Atrazine, Phorate, Trifluralin, Alachlor, 2.4D) and, as control, an area of the Tolfa district with alfalfa cultivations never treated with pesticides or chemical manures.

This first part of the research describes an application of the micronucleus test on fetuses in order to evaluate the mutagenetic risk to which they are exposed. On the fetuses the analysis of the primary ossification centers was also performed in order to determine their age, because it is known that foetal hepatic erythropoiesis in mice begins during the 10th day of gestation and is active until just before birth (Cole & Paul, 1966).

The micronucleus test, originally developed in bone marrow cells, is a sensitive method to detect cytogenetic damages in mammalian somatic cells in vivo; its usefulness in the detection of genetic effects of chemicals and radiations has been widely confirmed by many authors (Boller & Schmid 1970; Schmid 1976, 1977; Heddle & Carrano, 1977; Watanabe et al. 1982; Heddle et al. 1983). This test has been applied to wild Rodent populations from URSS areas contaminated with radium and uranium (Materij & Maslova, 1978) and to wild murine populations from nuclear power plant areas (Cristaldi et al. 1985) and from industrial, agricultural and urban polluted areas (Ieradi et al., 1984).

The transplacental micronucleus test, applied to the fetuses of rats and mice, provides a short term assay which is an appropriate solution to the problem of prenatal risk assessment (Cole et al. 1979; 1981; 1983; Stoyel & Clark, 1980; Jenssen & Ramel, 1980; Cihak & Vontorkova, 1985) and that so far has not been carried out on wild murine populations fetuses.

Materials and methods.

Trappings were carried out from June to August 1983. The specimens trapped were: n. 17 *Mus domesticus*, n. 2 *Rattus rattus*, n. 1 *Rattus norvegicus*, n. 1 *Apodemus sylvaticus* at Maccarese; n. 12 *Mus domesticus*, n. 2 *Rattus rattus*, n. 8 *Apodemus sylvaticus* at Tolfa. The animals were trapped using Havahart and Sherman live traps with choice quality baits. The micronucleus test was applied on two pregnant females from Maccarese (MAC 6 *M. domesticus* and MAC 11 *Apodemus sylvaticus*) and on their respective fetuses and on three pregnant females from Tolfa (CON 3 *A. sylvaticus*, CON 18 and CON 22 *M. domesticus*) and on their respective fetuses.

Micronucleus test

Single cell suspensions of fetal liver and adult femoral bone marrow were prepared in freshly filtered Hank's balanced salt solution + 5% fetal calf serum and smeared on pre-cleaned slides. Slides were air dried, fixed in absolute methanol and stained with May Grunwald (Merck) 0.25% in methanol for 3 min.; May Grunwald (1 : 1) with Sorensen buffer pH 6.8 for 2 min. and Giemsa (Merck) 14% in Sorensen buffer pH 6.7 for 1 min. Slides were briefly rinsed in buffer and then in distilled water, air dried and mounted in Permount. All solutions were filtered through Whatman GFC immediately before use. With this method the cytoplasm of polychromatic erythrocytes is blue stained, the cytoplasm of mature erythrocytes is yellow-orange stained and the micronuclei are red-purple stained (Cole et al. 1979); 1000 polychromatic erythrocytes per pregnant female and 1000 per each fetus were examined for the presence of micronuclei.

Determination of the age of fetuses.

In order to determinate the gestational age original data (Caronna & Parisi 1983; Caronna & Parisi in press) and data from other species (Ozdzénski & Mystkowska 1976; Sterba 1976; Edwards 1968; Zsjda 1968; Rinaldi et al. 1980; Caronna 1984) and from laboratory strains (Rinaldi 1968, 1969; Theiler 1972) were utilized. The embryos and fetuses, individually marked, depending on their position in the uterine horns, were observed with a light microscope for external morphology; they were subsequently stained with red-Alizarine and clarified with alcool and glycerine for reading the primary ossification centers.

Results and discussion.

The age determination of the specimens studied provided the following results:

MAC 6: *Mus domesticus* - n. 7 fetuses - gestational age: 17 days. Interparietal: fusion of the two ossification centers. Para-occipital: formation of the notch. Supra-occipital: two ossification centers fused in one. Mandible: formation of the alveolar process. Tympanic bulla: presence of an internal ossification center. Hyoid bone: presence of the central little bar. Spinal column: presence of the ossification center to the 2nd and the 3rd caudal vertebral level. Sternum: presence of one or two ossification centers to the 5th sternebra level.

MAC 11: *Apodemus sylvaticus* - n. 6 fetuses - gestational age 16 days. Nasal bones: presence of the anterior processes. Squamosal: presence of the osseous lamina. Supra-occipital: presence of the two ossification centers. Mandible: presence of the coronoid process. Vertebral column: neural arch present up to the 2nd and the 3rd sacral vertebra. Vertebral bodies: present up to the second sacral vertebra. Sternum: the ossification is beginning from the 1st to the 4th sternebra; in almost all the specimens the 6th is present to; the sternebrae from the 2nd to the 4th and the 6th are constituted by two ossification centers each.
Anterior limbs: presence of the collar of compact bone sorrounding cartilage of the diaphysis of the 3rd, 4th and 5th metacarpal bone.
Posterior limbs: presence of the collar of compact bone sorrounding cartilage of the diaphysis of the 3rd and the 4th metatarsal bone.

CON 3: *Apodemus sylvaticus* - n. 5 fetuses - gestational age: 12 days.
Age determination from the external morphology:
Emispheres of the encephalon: prominent. Eye: pigmentation of the iris. Hair follicles present in some areas of the body. Anterior limbs: « hand plates » flattened with finger rays and presence of the interdigital membrane.

CON 18: n. 5 fetuses, CON 22: n. 6 fetuses - *Mus domesticus* - gestational age: 11/12 days.
Age determination from the external morphology:
Tail: presence of the gemmula. Skin: lightly tight with few areas of hair follicles.
Eye: presence of the palpebral fissure. Anterior and posterior limbs: « hand-plates » and « foot-plates » flattened with evident interdigital membrane.

TABLE I. — Presence of micronuclei (MN) in polychromatic erythrocytes (PCE) of bone marrow and fetal liver of specimens from Maccarese and Tolfa. (L = legt; R = right)

Samples	Age	Species	PCE with MN/1000 PCE
MAC 6	adult	*Mus domesticus*	4 (bone marrow)
— 1L	fetus 17 days	*Mus domesticus*	4 (fetal liver)
— 2L	fetus 17 days	*Mus domesticus*	7 (fetal liver)
— 3L	fetus 17 days	*Mus domesticus*	8 (fetal liver)
— 4L	fetus 17 days	*Mus domesticus*	2 (fetal liver)
— 5L	fetus 17 days	*Mus domesticus*	2 (fetal liver)
— 1R	fetus 17 days	*Mus domesticus*	3 (fetal liver)
— 2R	fetus 17 days	*Mus domesticus*	1 (fetal liver)
MAC 11	adult	*Apodemus sylvaticus*	6 (bone marrow)
— 1L	fetus 16 days	*Apodemus sylvaticus*	7 (fetal liver)
— 2L	fetus 16 days	*Apodemus sylvaticus*	5 (fetal liver)
— 1R	fetus 16 days	*Apodemus sylvaticus*	2 (fetal liver)
— 2R	fetus 16 days	*Apodemus sylvaticus*	6 (fetal liver)
— 3R	fetus 16 days	*Apodemus sylvaticus*	5 (fetal liver)
— 4R	fetus 16 days	*Apodemus sylvaticus*	8 (fetal liver)
CON 3	adult	*Apodemus sylvaticus*	1 (bone marrow)
— 1L	fetus 12 days	*Apodemus sylvaticus*	0 (fetal liver)
— 1R	fetus 12 days	*Apodemus sylvaticus*	0 (fetal liver)
— 2R	fetus 12 days	*Apodemus sylvaticus*	0 (fetal liver)
— 3R	fetus 12 days	*Apodemus sylvaticus*	0 (fetal liver)
— 4R	fetus 12 days	*Apodemus sylvaticus*	0 (fetal liver)
CON 18	adult	*Mus domesticus*	0 (bone marrow)
— 1L	fetus 11-12 days	*Mus domesticus*	0 (fetal liver)
— 2L	fetus 11-12 days	*Mus domesticus*	0 (fetal liver)
— 3L	fetus 11-12 days	*Mus domesticus*	0 (fetal liver)
— 1R	fetus 11-12 days	*Mus domesticus*	0 (fetal liver)
— 2R	fetus 11-12 days	*Mus domesticus*	0 (fetal liver)
CON 22	adult	*Mus domesticus*	1 (bone marrow)
— 1L	fetus 11-12 days	*Mus domesticus*	0 (fetal liver)
— 2L	fetus 11-12 days	*Mus domesticus*	0 (fetal liver)
— 3L	fetus 11-12 days	*Mus domesticus*	0 (fetal liver)
— 1R	fetus 11-12 days	*Mus domesticus*	1 (fetal liver)
— 2R	fetus 11-12 days	*Mus domesticus*	0 (fetal liver)
— 3R	fetus 11-12 days	*Mus domesticus*	0 (fetal liver)

The results obtained, even though partial, applying the transplacental micronucleus test (see Tab. 1) show a significant presence of micronucleated polycromatic erythrocytes in the specimens from Maccarese site compared to the specimens from the control area (Tolfa site).

Considering the test reliability and its large application in the detection of the pesticide mutagenicity in rats and mice (AMHER & FAHMY 1982; 1983; DOULOUT et al. 1982a, 1982b; AMER & ABOUL-ELA 1985; GROVER & MAHLI 1985) these preliminary results provide an important indication that prenatal damages can be induced in wild murine populations living in areas treated with pesticides.

These data confirm the importance to test an higher number of samples from the sites studied and to carry out a continuous monitoring in order to evaluate the damage and its frequence. Moreover the limits of the experiments on wild animals, as biological indicators, should be considered, as they present an higher inter-individual variability and can be genetically more protected than the laboratory strains which are used in mutagenicity testing.

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